Please amend the specification as follows:

Please replace the paragraph beginning at page 2, line 7, with the following paragraph:

Although the ATP-dependent ligases and NAD^+-dependent ligases share little sequence homology, all the ligases investigated so far use the same KXDG (SEQ. ID. No. 24) motif to form adenylated enzyme intermediate (Tomkinson, et al., Bioessays, 19(10):893-901 (1997), Shuman, et al., Virology, 211(1):73-83 (1995), and Luo, et al., Nucleic Acids Res, 24(15):3079-3085 (1996)). Furthermore, they seem to be organized by similar domains and structural folds ((Doherty, et al., Nucleic Acids Res, 24(12):2281-2287 (1996), Subramanya, et al., Cell, 85(4):607-615 (1996), and Sekiguchi, et al., Nucleic Acids Res, 25(4):727-734 (1997)). The diversity of ligase sequences is not only reflected by their different optimal reaction conditions and kinetic rates, but more importantly by their different specificities toward match and mismatch substrates. Among the viral ATP-dependent ligases, the broad substrate tolerance is represented by the T4 enzyme which seals various mismatches on both the 3' and 5' side of the nick junction (Wu, et al., Gene, 76(2):245-254 (1989)). Vaccinia ligase ligates various mismatches at both 3'-hydroxyl or 5'-phosphate sides with the exception of purine-purine mismatch pairs at the 3'-hydroxyl side (Shuman, S., Biochemistry, 34(49):16138-161475 (1995)). Mammalian ATP-dependent ligases show different substrate sensitivity, as ligase I is more sensitive to 3' mismatches than ligase III (Husain, et al., J Biol Chem, 270(16):9683-9690 (1995)). Additionally, both ligase I and III tolerate a 3'C/T mismatch more than a 3'G/T mismatch. Little is known about archeal ATP-dependent ligases which may reveal the nature of the progenitor of ATP-dependent ligases. Studies on NAD^+-dependent DNA ligase from *E. coli*, along with T4 ligase, have contributed immensely to understanding of the basic biochemical pathway of the DNA ligation reaction (Lehman, I.R., Science, 186(4166):790-797 (1974) and Rossi, et al., Nucleic Acids Res, 25(11):2106-2113 (1997)). Studies on the NAD^+-dependent ligase from *Thermus thermophilus* HB8 have revealed the highly discriminative power this enzyme possesses (Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996)). Although mismatches at 5'-phosphate side are tolerated to some degree (5'A/C, 5'A/A, 5'C/A, 5'C/T, 5'G/T, 5'G/A, 5'T/T, 5'T/G), mismatches at the 3'-hydroxyl side essentially abolish nick-closure activity except 3'G/T or 3'T/G mismatch (Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996)). Apparently, sequence divergence and subsequent subtle structural variation among DNA ligases underlie an enzyme's recognition preferences toward different mismatched base-pairs.

Please replace the paragraph beginning at page 7, line 13, with the following paragraph:

Figures 1A-C show a sequence comparison of *Thermus* DNA ligases. Figure 1A illustrates the evolutionary tree for *Thermus* DNA ligases. Figure 1B is a regional sequence alignment of nine *Thermus* ligases: *Tsp.* AK16D (SEQ. ID. No. 22); *Thermus aquaticus* YT-1 (SEQ. ID. No. 15); *Thermus Thermophilus ("Tth")* (SEQ. ID. No. 23); *Thermus flavus* (SEQ. ID. No. 16); *Thermus filiformis* Tok4A2 (SEQ. ID. No. 17); *Thermus filiformis* Tok6A1 (SEQ. ID. No. 18); *Tsp.* SM32 (SEQ. ID. No. 19); *Tsp.* Vil3 (SEQ. ID. No. 20); *T. scot* (SEQ. ID. No. 21). The aa (i.e. amino acid) sequence of ~~T. scot~~ *T. scot* ~~is~~ retrieved from ~~Genebank~~ GenBank by accession number 1085749 (SEQ. ID. No. 31), is as follows:

```
Met Thr Leu Glu Glu Ala Arg Lys Arg Val Asn Glu Leu Arg Asp Leu
 1               5                  10                  15

Ile Arg Tyr His Asn Tyr Arg Tyr Tyr Val Leu Ala Asp Pro Glu Ile
            20                  25                  30

Ser Asp Ala Glu Tyr Asp Arg Leu Leu Arg Glu Leu Lys Glu Leu Glu
        35                  40                  45

Glu Arg Phe Pro Glu Leu Lys Ser Pro Asp Ser Pro Thr Glu Gln Val
    50                  55                  60

Gly Ala Lys Pro Leu Glu Ala Thr Phe Arg Pro Ile Arg His Pro Thr
65                  70                  75                  80

Arg Met Tyr Ser Leu Asp Asn Ala Phe Asn Phe Asp Glu Leu Lys Ala
                85                  90                  95

Phe Glu Glu Arg Ile Gly Arg Ala Leu Gly Arg Glu Gly Pro Phe Ala
            100                 105                 110

Tyr Thr Val Glu His Lys Val Asp Gly Leu Ser Val Asn Leu Tyr Tyr
        115                 120                 125

Glu Asp Gly Val Leu Val Trp Gly Ala Thr Arg Gly Asp Gly Glu Val
    130                 135                 140

Gly Glu Glu Val Thr Gln Asn Leu Leu Thr Ile Pro Thr Ile Pro Arg
145                 150                 155                 160

Arg Val Lys Gly Val Pro Glu Arg Leu Glu Val Arg Gly Glu Val Tyr
                165                 170                 175
```

Met Pro Ile Glu Ala Phe Leu Arg Leu Asn Glu Glu Leu Glu Glu Lys
180 185 190

Gly Glu Lys Ile Phe Lys Asn Pro Arg Asn Ala Ala Ala Gly Ser Leu
195 200 205

Arg Gln Lys Asp Pro Arg Ile Thr Ala Arg Arg Gly Leu Arg Ala Thr
210 215 220

Phe Tyr Ala Leu Gly Leu Gly Leu Glu Glu Ser Gly Leu Lys Thr Gln
225 230 235 240

Leu Asp Leu Leu His Trp Leu Arg Glu Lys Gly Phe Pro Val Glu His
245 250 255

Gly Phe Ala Arg Ala Glu Gly Ala Glu Gly Val Glu Arg Ile Tyr Gln
260 265 270

Gly Trp Leu Lys Glu Arg Arg Ser Leu Pro Phe Glu Ala Asp Gly Val
275 280 285

Val Val Lys Leu Asp Glu Leu Ser Leu Trp Arg Glu Leu Gly Tyr Thr
290 295 300

Ala Arg Ala Pro Arg Phe Ala Ile Ala Tyr Lys Phe Pro Ala Glu Glu
305 310 315 320

Lys Glu Thr Arg Leu Leu Gln Val Val Phe Gln Val Gly Arg Thr Gly
325 330 335

Arg Val Thr Pro Val Gly Ile Leu Glu Pro Val Phe Ile Glu Gly Ser
340 345 350

Val Val Ser Arg Val Thr Leu His Asn Glu Ser Tyr Ile Glu Glu Leu
355 360 365

Asp Val Arg Ile Gly Asp Trp Val Leu Val His Lys Ala Gly Gly Val
370 375 380

Ile Pro Glu Val Leu Arg Val Leu Lys Glu Lys Arg Thr Gly Glu Glu
385 390 395 400

Arg Pro Ile Arg Trp Pro Glu Thr Cys Pro Glu Cys Gly His Arg Leu
405 410 415

Val Lys Glu Gly Lys Val His Arg Cys Pro Asn Pro Leu Cys Pro Ala
420 425 430

Lys Arg Phe Glu Ala Ile Arg His Tyr Ala Ser Arg Lys Ala Met Asp
435 440 445

Ile Gly Gly Leu Gly Glu Lys Leu Ile Glu Lys Leu Leu Glu Lys Gly
450 455 460

```
Leu Val Lys Asp Val Ala Asp Leu Tyr Arg Leu Lys Lys Glu Asp Leu
465                 470                 475                 480

Leu Gly Leu Glu Arg Met Gly Glu Lys Ser Ala Gln Asn Leu Leu Arg
                485                 490                 495

Gln Ile Glu Glu Ser Lys Gly Arg Gly Leu Glu Arg Leu Leu Tyr Ala
            500                 505                 510

Leu Gly Leu Pro Gly Val Gly Glu Val Leu Ala Arg Asn Leu Ala Ala
        515                 520                 525

His Phe Gly Thr Met Asp Arg Leu Leu Glu Ala Ser Leu Glu Glu Leu
    530                 535                 540

Leu Gln Val Glu Glu Val Gly Glu Leu Thr Ala Arg Gly Ile Tyr Glu
545                 550                 555                 560

Thr Leu Gln Asp Pro Ala Phe Arg Asp Leu Val Arg Arg Leu Lys Glu
                565                 570                 575

Ala Gly Val Val Met Glu Ala Lys Glu Arg Gly Glu Glu Ala Leu Lys
            580                 585                 590

Gly Leu Thr Phe Val Ile Thr Gly Glu Leu Ser Arg Pro Arg Glu Glu
        595                 600                 605

Val Lys Ala Leu Leu Arg Arg Leu Gly Ala Lys Val Thr Asp Ser Val
    610                 615                 620

Ser Arg Lys Thr Ser Tyr Leu Val Val Gly Glu Asn Pro Gly Ser Lys
625                 630                 635                 640

Leu Glu Lys Ala Arg Ala Leu Gly Val Pro Thr Leu Thr Glu Glu Glu
                645                 650                 655

Leu Tyr Arg Leu Ile Glu Glu Arg Thr Gly Lys Pro Val Glu Thr Leu
            660                 665                 670

Ala Ser.
```

The adenylation motif KXDG (SEQ. ID. No. 24) is underlined and the adenylation site is marked by *. The numbering of aa is based on *Tsp.* AK16D ligase (SEQ. ID. No. 1. Figure 1C is a complete amino acid sequence of *Tsp.* AK16D ligase (SEQ. ID. No. 1). The adenylation motif KXDG (SEQ. ID. No. 24) is underlined and the adenylation site ^{118}K is shown with a (*) above the residue. The complete sequence of *Tsp.* AK16D ligase gene and partial sequences of six other *Thermus* ligase genes have been deposited with GenBank under accession No. AF092862 for *Tsp.* AK16D (SEQ. ID. No. 1), AF092863 for *Thermus aquaticus* YT-1 (SEQ. ID. No. 25), as follows:

Pro	Glu	Leu	Lys	Ser	Pro	Asp	Ser	Pro	Thr	Glu	Gln	Val	Gly	Ala	Arg
1				5					10						15

Pro	Leu	Glu	Ser	Thr	Phe	Arg	Pro	Val	Arg	His	Pro	Thr	Arg	Met	Tyr
			20					25					30		

Ser	Leu	Asp	Asn	Ala	Phe	Ser	Leu	Asp	Glu	Val	Arg	Ala	Phe	Glu	Glu
		35					40					45			

Arg	Ile	Glu	Arg	Ala	Leu	Gly	Arg	Lys	Gly	Pro	Phe	Leu	Tyr	Thr	Val
	50					55					60				

Glu	His	Lys	Val	Asp	Gly	Leu	Ser	Val	Asn	Leu	Tyr	Tyr	Glu	Glu	Gly
65					70					75					80

Ile	Leu	Val	Phe	Gly	Ala	Thr	Arg	Gly	Asp	Gly	Glu	Thr	Gly	Glu	Glu
				85					90					95	

Val	Thr	Gln	Asn	Leu	Leu	Thr	Ile	Arg	Thr	Ile	Pro	Arg	Arg	Leu	Thr
			100					105					110		

Gly	Val	Pro	Asp	Arg	Leu	Glu	Val	Arg	Gly	Glu	Val	Tyr	Met	Pro	Ile
		115					120					125			

Glu	Ala	Phe	Leu	Arg	Leu	Asn	Gln	Glu	Leu	Glu	Glu	Ala	Gly	Glu	Arg
	130					135					140				

Ile	Phe	Lys	Asn	Pro	Arg	Asn	Ala	Ala	Ala	Gly	Ser	Leu	Arg	Gln	Lys
145					150					155					160

Asp	Pro	Arg	Val	Thr	Ala	Arg	Arg	Gly	Leu	Arg	Ala	Thr	Phe	Tyr	Ala
					165					170					175

Leu	Gly	Leu	Gly	Leu	Glu	Glu	Thr	Gly	Leu	Lys	Ser	Gln	His	Asp	Leu
			180					185					190		

Leu	Leu	Trp	Leu	Lys	Glu	Arg	Gly	Phe	Pro	Val	Glu	His	Gly	Phe	Thr
		195					200					205			

Arg	Ala	Leu	Gly	Ala	Glu	Gly	Val	Glu	Glu	Val	Tyr	Gln	Ala	Trp	Leu
	210					215					220				

Lys	Glu	Arg	Arg	Lys	Leu	Pro	Phe	Glu	Ala	Asp	Gly	Val	Val	Val	Lys
225					230					235					240

Leu	Asp	Asp	Leu	Ala	Leu	Trp	Arg	Glu	Leu	Gly	Tyr	Thr	Ala	Arg	Ala
					245					250					255

Pro	Arg	Phe	Ala	Leu	Ala	Tyr	Lys	Phe	Pro	Ala	Glu	Glu	Lys	Glu	Thr
			260					265					270		

Arg	Leu	Leu	Ser	Val	Ala	Phe	Gln	Val	Gly	Arg	Thr	Gly	Arg	Ile	Thr
		275					280					285			

```
Pro Val Gly Val Leu Glu Pro Val Phe Ile Glu Gly Ser Glu Val Ser
    290                 295                 300

Arg Val Thr Leu His Asn Glu Ser Phe Ile Glu Glu Leu Asp Val Arg
305                 310                 315                 320

Ile Gly Asp Trp Val Leu Val His Lys Ala Gly Gly Val Ile Pro Glu
                325                 330                 335

Val Leu Arg Val Leu Lys Glu Arg Arg Thr Gly Glu Glu Lys Pro Ile
            340                 345                 350

Leu Trp Pro Glu Asn Cys Pro Glu Cys Gly His Ala Leu Leu Lys Glu
        355                 360                 365

Gly Lys Val His Arg Cys Pro Asn Pro Leu Cys Pro Ala Lys Arg Phe
    370                 375                 380

Glu Ala Ile Arg His Tyr Ala Ser Arg Lys Ala Met Asp Ile Gln Gly
385                 390                 395                 400

Leu Gly Glu Lys Leu Ile Glu Lys Leu Leu Glu Lys Gly Leu Val Arg
                405                 410                 415

Asp Val Ala Asp Leu Tyr Arg Leu Arg Lys Glu Asp Leu Leu Asp Leu
            420                 425                 430

Glu Arg Met Gly Glu Lys Ser Ala Glu Asn Leu Leu Arg Gln Ile Glu
        435                 440                 445

Glu Ser Lys Gly Arg Gly Leu Glu Arg Leu Leu Tyr Ala Leu Gly Leu
    450                 455                 460

Pro Gly Val Gly Glu Val Leu Ala Arg Asn Leu Ala Leu Arg Phe Gly
465                 470                 475                 480

His Met Asp Arg Leu Leu Glu Ala Gly Leu Gly Asp Leu Leu Glu Val
                485                 490                 495

Glu Gly Val Gly Glu Leu Thr Ala Arg Ala Ile Leu Asn Thr Leu Lys
            500                 505                 510

Asp Pro Glu Phe Arg Asp Leu Val Arg Arg Leu Lys Glu Ala Gly
        515                 520                 525        ;
```

AF092864 for *Thermus flavus* (SEQ. ID. No. 26), as follows:

```
Arg Phe Pro Glu Leu Lys Ser Pro Asp Ser Pro Thr Glu Gln Val Gly
  1                 5                  10                  15

Ala Arg Pro Leu Glu Ala Thr Phe Arg Pro Val Arg His Pro Thr Arg
            20                  25                  30
```

Met Tyr Ser Leu Asp Asn Ala Phe Asn Phe Asp Glu Leu Lys Ala Phe
35 40 45

Glu Glu Arg Ile Glu Arg Ala Leu Gly Arg Glu Gly Pro Phe Ala Tyr
50 55 60

Thr Val Glu His Lys Val Asp Gly Leu Ser Val Asn Leu Tyr Tyr Glu
65 70 75 80

Asp Gly Val Leu Val Tyr Gly Ala Thr Arg Gly Asp Gly Glu Val Gly
85 90 95

Glu Glu Val Thr Gln Asn Leu Leu Thr Ile Pro Thr Ile Pro Arg Arg
100 105 110

Leu Lys Gly Val Pro Glu Arg Leu Glu Val Arg Gly Glu Val Tyr Met
115 120 125

Pro Val Glu Ala Phe Leu Arg Leu Asn Glu Glu Leu Glu Glu Arg Gly
130 135 140

Ala Arg Ile Phe Lys Asn Pro Arg Asn Ala Ala Ala Gly Ser Leu Arg
145 150 155 160

Gln Lys Asp Pro Arg Ile Thr Ala Lys Arg Gly Leu Arg Ala Thr Phe
165 170 175

Tyr Ala Leu Gly Leu Gly Leu Glu Glu Val Glu Arg Glu Gly Val Ala
180 185 190

Thr Gln Phe Ala Leu Leu His Trp Leu Lys Glu Lys Ser Phe Pro Val
195 200 205

Glu His Gly Tyr Ala Arg Ala Val Gly Ala Glu Gly Val Glu Ala Val
210 215 220

Tyr Gln Asp Trp Leu Lys Lys Arg Arg Ala Leu Pro Phe Glu Ala Asp
225 230 235 240

Gly Val Val Val Lys Leu Asp Glu Leu Ala Leu Trp Arg Glu Leu Gly
245 250 255

Tyr Thr Ala Arg Ala Pro Arg Phe Ala Ile Ala Tyr Lys Phe Pro Ala
260 265 270

Glu Glu Lys Glu Thr Arg Leu Leu Asp Val Ala Phe Gln Val Gly Arg
275 280 285

Thr Gly Arg Val Thr Pro Val Gly Ile Leu Glu Pro Val Phe Leu Glu
290 295 300

Gly Ser Glu Val Ser Arg Val Thr Leu His Asn Glu Ser Tyr Ile Glu
305 310 315 320

```
Glu Leu Asp Ile Arg Ile Gly Asp Trp Val Leu Val His Lys Ala Gly
                325                 330                 335

Gly Val Ile Pro Glu Val Leu Arg Val Leu Lys Glu Arg Arg Thr Gly
            340                 345                 350

Glu Glu Arg Pro Ile Arg Trp Pro Glu Thr Cys Pro Glu Cys Gly His
        355                 360                 365

Arg Leu Leu Lys Glu Gly Lys Val His Arg Cys Pro Asn Pro Leu Cys
    370                 375                 380

Pro Ala Lys Arg Phe Glu Ala Ile Arg His Phe Pro Ser Arg Lys Ala
385                 390                 395                 400

Met Asp Ile Gln Gly Leu Gly Glu Lys Leu Ile Glu Arg Leu Leu Glu
                405                 410                 415

Lys Gly Leu Val Lys Asp Val Ala Asp Leu Tyr Arg Leu Arg Lys Glu
            420                 425                 430

Asp Leu Val Gly Leu Glu Arg Met Gly Glu Lys Ser Ala Gln Asn Leu
        435                 440                 445

Leu Arg Gln Ile Glu Glu Ser Lys Arg Arg Gly Leu Glu Arg Leu Leu
    450                 455                 460

Tyr Ala Leu Gly Leu Pro Gly Val Gly Glu Val Leu Ala Arg Asn Leu
465                 470                 475                 480

Ala Ala Arg Phe Gly Asn Met Asp Arg Leu Leu Glu Ala Ser Leu Glu
                485                 490                 495

Glu Leu Leu Glu Val Glu Glu Val Gly Glu Leu Thr Ala Arg Ala Ile
            500                 505                 510

Leu Glu Thr Leu Lys Asp Pro Ala Phe Arg Asp Leu Val Arg Arg Leu
        515                 520                 525

Lys Glu Ala Gly Val Glu Met Glu Ala Lys Glu Lys Gly Gly Glu Ala
    530                 535                 540

Leu Lys Gly Leu Thr Phe Val Ile Thr Gly Glu Leu Ser
545                 550                 555         ;
```

AF092865 for *Thermus filiformis* Tok4A2 (SEQ. ID. No. 27), as follows:

```
Asp Ser Pro Thr Glu Gln Val Gly Ala Arg Pro Leu Glu Pro Thr Phe
 1               5                  10                  15

Arg Pro Val Arg His Pro Thr Arg Met Tyr Ser Leu Asp Asn Ala Phe
            20                  25                  30
```

Thr Tyr Glu Glu Val Leu Ala Phe Glu Glu Arg Leu Asp Arg Ala Leu
35 40 45

Gly Arg Lys Arg Pro Phe Leu Tyr Thr Val Glu His Lys Val Asp Gly
50 55 60

Leu Ser Val Asn Leu Tyr Tyr Glu Glu Gly Val Leu Val Phe Gly Ala
65 70 75 80

Thr Arg Gly Asp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

Thr Ile Pro Thr Ile Pro Arg Arg Leu Lys Gly Val Pro Asp Arg Leu
100 105 110

Glu Val Arg Gly Glu Val Tyr Met Pro Ile Glu Ala Phe Leu Arg Leu
115 120 125

Asn Glu Glu Leu Glu Glu Arg Gly Glu Lys Val Phe Lys Asn Pro Arg
130 135 140

Asn Ala Ala Ala Gly Ser Leu Arg Gln Lys Asp Pro Arg Val Thr Ala
145 150 155 160

Lys Arg Gly Leu Arg Ala Thr Phe Tyr Ala Leu Gly Leu Gly Leu Glu
165 170 175

Glu Ser Gly Leu Lys Ser Gln Tyr Glu Leu Leu Leu Trp Leu Lys Glu
180 185 190

Lys Gly Phe Pro Val Glu His Gly Tyr Glu Lys Ala Leu Gly Ala Glu
195 200 205

Gly Val Glu Glu Val Tyr Gln Ala Xaa Xaa Xaa Lys Arg His Ala Leu
210 215 220

Pro Phe Glu Ala Asp Gly Val Val Val Lys Met Asp Asp Leu Thr Leu
225 230 235 240

Trp Gly Glu Leu Gly Tyr Thr Ala Arg Ala Pro Arg Phe Ala Ile Ala
245 250 255

Tyr Lys Phe Pro Ala Glu Glu Asn Glu Thr Arg Leu Leu Asp Val Asp
260 265 270

Phe Gln Val Gly Arg Thr Gly Arg Val Thr Pro Val Gly Ile Leu Glu
275 280 285

Pro Val Phe Leu Glu Gly Ser Glu Val Ser Arg Val Thr Leu His Asn
290 295 300

Glu Ser Tyr Ile Glu Glu Leu Asp Ile Arg Ile Gly Asp Trp Val Leu
305 310 315 320

```
Val His Lys Ala Gly Gly Val Ile Pro Glu Val Leu Arg Val Leu Lys
                  325                 330                 335

Glu Arg Arg Thr Gly Glu Glu Arg Pro Ile Arg Trp Pro Glu Thr Cys
              340                 345                 350

Pro Glu Cys Gly His Arg Leu Leu Lys Glu Gly Lys Val His Arg Cys
          355                 360                 365

Pro Asn Pro Leu Cys Pro Ala Lys Arg Phe Glu Ala Ile Arg His Phe
      370                 375                 380

Pro Ser Arg Lys Ala Met Asp Ile Gln Gly Leu Gly Glu Lys Leu Ile
385                 390                 395                 400

Glu Arg Leu Leu Glu Lys Gly Leu Val Lys Asp Val Ala Asp Leu Tyr
                  405                 410                 415

Arg Leu Arg Lys Glu Asp Leu Val Gly Leu Glu Arg Met Gly Glu Lys
              420                 425                 430

Ser Ala Gln Asn Leu Leu Arg Gln Ile Glu Glu Ser Lys Arg Arg Gly
          435                 440                 445

Leu Glu Arg Leu Leu Tyr Ala Leu Gly Leu Pro Gly Val Gly Glu Val
      450                 455                 460

Leu Ala Arg Asn Leu Ala Ala Arg Phe Gly Asn Met Asp Arg Leu Leu
465                 470                 475                 480

Glu Ala Ser Leu Glu Glu Leu Leu Glu Val Glu Glu Val Gly Glu Leu
                  485                 490                 495

Thr Ala Arg Ala Ile Leu Glu Thr Leu Lys Asp Pro Ala Phe Arg Asp
              500                 505                 510

Leu Val Arg Arg Leu Lys Glu Ala Gly Val Glu Met Glu Ala Lys Glu
          515                 520                 525

Lys Gly Gly Glu Ala Leu Lys Gly Leu Thr Phe Val Ile Thr Gly Glu
      530                 535                 540

Leu Ser
545     ;
```

AF092866 for *Thermus filiformis* Tok6A1 (SEQ. ID. No. 28), as follows:

```
Arg Phe Pro Glu Phe Lys Ser Pro Asp Ser Pro Thr Glu Gln Val Gly
  1                 5                  10                  15

Ala Arg Pro Leu Glu Pro Thr Phe Arg Pro Val Arg His Pro Thr Arg
              20                  25                  30
```

```
Met Tyr Ser Leu Asp Asn Ala Phe Thr Tyr Glu Glu Val Leu Ala Phe
          35                  40                  45

Glu Glu Arg Leu Glu Arg Ala Leu Gly Arg Lys Arg Pro Phe Leu Tyr
     50                  55                  60

Thr Val Glu His Lys Val Asp Gly Leu Ser Val Asn Leu Tyr Tyr Glu
 65                  70                  75                  80

Glu Gly Val Leu Val Phe Gly Ala Thr Arg Gly Asp Gly Glu Val Gly
                 85                  90                  95

Glu Glu Val Thr Gln Asn Leu Leu Thr Ile Pro Thr Ile Pro Arg Arg
            100                 105                 110

Leu Lys Gly Val Pro Asp Arg Leu Glu Val Arg Gly Glu Val Tyr Met
        115                 120                 125

Pro Ile Glu Ala Phe Leu Arg Leu Asn Glu Glu Leu Glu Glu Arg Gly
    130                 135                 140

Glu Lys Val Phe Lys Asn Pro Arg Asn Ala Ala Ala Gly Ser Leu Arg
145                 150                 155                 160

Gln Lys Asp Pro Arg Val Thr Ala Lys Arg Gly Leu Arg Ala Thr Phe
                165                 170                 175

Tyr Ala Leu Gly Leu Gly Leu Glu Glu Ser Gly Leu Lys Ser Gln Tyr
            180                 185                 190

Glu Leu Leu Leu Trp Leu Lys Glu Lys Gly Phe Pro Val Glu His Gly
        195                 200                 205

Tyr Glu Lys Ala Leu Gly Ala Glu Gly Val Glu Glu Val Tyr Arg Arg
    210                 215                 220

Phe Leu Ala Gln Arg His Ala Leu Pro Phe Glu Ala Asp Gly Val Val
225                 230                 235                 240

Val Lys Leu Asp Asp Leu Ala Leu Trp Arg Glu Leu Gly Tyr Thr Ala
                245                 250                 255

Arg Ala Pro Arg Phe Ala Leu Ala Tyr Lys Phe Pro Ala Glu Glu Lys
            260                 265                 270

Glu Thr Arg Leu Leu Asp Val Val Phe Gln Val Gly Arg Thr Gly Arg
        275                 280                 285

Val Thr Pro Val Gly Val Leu Glu Pro Val Phe Ile Glu Gly Ser Glu
    290                 295                 300

Val Ser Arg Val Thr Leu His Asn Glu Ser Tyr Ile Glu Glu Leu Asp
305                 310                 315                 320

Ile Arg Ile Gly Asp Trp Val Leu Val His Lys Ala Gly Gly Val Ile
```

```
                325                 330                 335

Pro Glu Val Leu Arg Val Leu Lys Glu Arg Arg Thr Gly Glu Glu Arg
            340                 345                 350

Pro Ile Arg Trp Pro Glu Thr Cys Pro Glu Cys Gly His Arg Leu Val
        355                 360                 365

Lys Glu Gly Lys Val His Arg Cys Pro Asn Pro Leu Cys Pro Ala Lys
    370                 375                 380

Arg Phe Glu Ala Ile Arg His Tyr Ala Ser Arg Lys Ala Met Asp Ile
385                 390                 395                 400

Glu Gly Leu Gly Glu Lys Leu Ile Glu Arg Leu Leu Glu Lys Gly Leu
                405                 410                 415

Val Arg Asp Val Ala Asp Leu Tyr His Leu Arg Lys Glu Asp Leu Leu
            420                 425                 430

Gly Leu Glu Arg Met Gly Glu Lys Ser Ala Gln Asn Leu Leu Arg Gln
        435                 440                 445

Ile Glu Glu Ser Lys His Arg Gly Leu Glu Arg Leu Leu Tyr Ala Leu
    450                 455                 460

Gly Leu Pro Gly Val Gly Glu Val Leu Ala Arg Asn Leu Ala Arg Arg
465                 470                 475                 480

Phe Gly Thr Met Asp Arg Leu Leu Glu Ala Ser Leu Glu Glu Leu Leu
                485                 490                 495

Glu Val Glu Glu Val Gly Glu Leu Thr Ala Arg Ala Ile Leu Glu Thr
            500                 505                 510

Leu Lys Asp Pro Ala Phe Arg Asp Leu Val Arg Arg Leu Lys Glu Ala
        515                 520                 525

Gly Val Ser Met Glu Ser Lys Glu Glu
    530                 535         ;
```

AF092867 for *Tsp.* Vil3 (SEQ. ID. No. 29), as follows:

```
Pro Ser Pro Asp Ser Pro Thr Glu Gln Val Gly Ala Lys Pro Leu Glu
  1                 5                  10                  15

Ala Thr Phe Arg Pro Ile Arg His Pro Thr Arg Met Tyr Ser Leu Asp
            20                  25                  30

Asn Ala Phe Thr Leu Glu Glu Val Arg Thr Phe Glu Glu Arg Ile Glu
        35                  40                  45
```

```
Arg Ala Leu Gly Arg Lys Gly Pro Phe Val Tyr Thr Val Glu His Lys
     50                  55                  60

Val Asp Gly Leu Ser Val Asn Leu Tyr Tyr Glu Glu Gly Ile Leu Val
 65                  70                  75                  80

Trp Gly Ala Thr Arg Gly Asp Gly Glu Thr Gly Glu Glu Val Thr Gln
                 85                  90                  95

Asn Leu Leu Thr Ile Pro Thr Ile Pro Arg Arg Leu Lys Gly Val Pro
            100                 105                 110

Glu Arg Leu Glu Val Arg Gly Glu Val Tyr Met Pro Ile Glu Ala Phe
        115                 120                 125

Leu Arg Leu Asn Glu Glu Leu Glu Glu Lys Gly Glu Lys Ile Phe Lys
    130                 135                 140

Asn Pro Arg Asn Ala Ala Ala Gly Ser Phe Arg Gln Lys Asp Pro Arg
145                 150                 155                 160

Ile Thr Ala Arg Arg Gly Leu Arg Ala Thr Phe Tyr Ala Leu Gly Leu
                165                 170                 175

Gly Leu Glu Glu Ser Gly Leu Lys Thr Gln Leu Asp Leu Leu His Trp
            180                 185                 190

Leu Arg Glu Lys Gly Phe Pro Val Glu His Gly Phe Ala Arg Ala Glu
        195                 200                 205

Gly Ala Glu Gly Val Glu Arg Ile Tyr Gln Gly Trp Leu Lys Glu Arg
    210                 215                 220

Arg Ser Leu Pro Phe Glu Ala Asp Gly Val Val Val Lys Leu Asp Glu
225                 230                 235                 240

Leu Ser Leu Trp Arg Glu Leu Gly Tyr Thr Ala Arg Ala Pro Arg Phe
                245                 250                 255

Ala Ile Ala Tyr Lys Phe Pro Ala Glu Glu Lys Glu Thr Ala Leu Phe
            260                 265                 270

Gln Val Val Leu Gln Val Gly Arg Thr Gly Gln Val Thr Pro Val Gly
        275                 280                 285

Ile Leu Glu Pro Val Phe Ile Glu Gly Ser Glu Val Ser Arg Val Thr
    290                 295                 300

Leu His Asn Glu Ser Tyr Ile Glu Asp Leu Asp Val Arg Ile Gly Glu
305                 310                 315                 320

Trp Val Leu Val His Asn Ala Gly Gly Val Ile Pro Glu Val Leu Arg
                325                 330                 335

Val Leu Lys Glu Lys Arg Thr Gly Glu Glu Arg Pro Ile Arg Trp Pro
```

```
                340                 345                 350

Glu Thr Cys Pro Glu Cys Gly His Arg Leu Val Lys Glu Gly Lys Val
        355                 360                 365

His Arg Cys Pro Asn Pro Leu Cys Pro Ala Lys Arg Phe Glu Ala Ile
    370                 375                 380

Arg His Tyr Ala Ser Arg Lys Ala Met Asp Ile Gly Gly Leu Gly Glu
385                 390                 395                 400

Lys Leu Ile Glu Lys Leu Leu Glu Lys Gly Leu Val Lys Asp Val Ala
                405                 410                 415

Asp Leu Tyr Arg Leu Lys Glu Glu Asp Leu Val Gly Leu Glu Arg Met
            420                 425                 430

Gly Lys Lys Ser Ala Gln Asn Leu Leu Arg Gln Ile Glu Lys Ser Lys
        435                 440                 445

Ala Arg Gly Leu Glu Arg Leu Leu Tyr Ala Leu Gly Leu Pro Gly Val
    450                 455                 460

Gly Glu Val Leu Ala Arg Asn Leu Ala Ala His Phe Gly Thr Met Asp
465                 470                 475                 480

Arg Leu Leu Glu Ala Ser Leu Glu Glu Leu Leu Gln Val Glu Glu Val
                485                 490                 495

Gly Glu Leu Thr Ala Arg Gly Ile Tyr
            500                 505;
```

and AF092868 for *Tsp.* SM32 (SEQ. ID. No. 30), as follows:

```
Asp Asn Ala Phe Thr His His Asp Leu Lys Ala Phe Glu Asp Arg Val
 1                  5                  10                  15

Asp Arg Ala Leu Gly Arg Glu Gly Pro Phe Val Tyr Thr Val Glu His
            20                  25                  30

Lys Val Asp Gly Leu Ser Val Asn Leu Tyr Tyr Glu Glu Gly Ile Leu
        35                  40                  45

Val Phe Gly Ala Pro Arg Gly Asp Gly Glu Val Gly Glu Glu Val Thr
    50                  55                  60

Gln Asn Leu Leu Thr Ile Pro Thr Ile Pro Arg Arg Leu Lys Gly Val
65                  70                  75                  80

Pro Glu Arg Leu Glu Val Arg Gly Glu Val Tyr Met Pro Ile Glu Ala
                85                  90                  95
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Phe Leu Arg Leu Asn Glu Glu Leu Glu Glu Ala Gly Glu Lys Val Phe
            100                 105                 110

Lys Asn Pro Arg Asn Ala Ala Ala Gly Ser Leu Arg Gln Lys Asp Pro
        115                 120                 125

Arg Ile Thr Ala Lys Arg Gly Leu Arg Ala Thr Phe Tyr Ala Leu Gly
    130                 135                 140

Leu Gly Leu Glu Glu Ser Gly Leu Lys Thr Gln Tyr Glu Phe Leu Leu
145                 150                 155                 160

Trp Phe Lys Glu Lys Gly Phe Pro Val Glu His Gly Phe Ala Arg Ala
                165                 170                 175

Thr Gly Ala Glu Gly Val Glu Arg Val Tyr Gln Gly Trp Leu Gln Lys
            180                 185                 190

Arg Arg Lys Leu Pro Phe Glu Ala Asp Gly Val Val Val Lys Leu Asp
        195                 200                 205

Glu Leu Ala Leu Trp Arg Glu Leu Gly Tyr Thr Ala Arg Ala Pro Arg
    210                 215                 220

Phe Ala Ile Ala Tyr Lys Phe Pro Ala Glu Glu Lys Glu Thr Arg Leu
225                 230                 235                 240

Leu Asp Val Val Phe Gln Val Gly Arg Thr Gly Arg Val Thr Pro Val
                245                 250                 255

Gly Ile Leu Glu Pro Val Leu Ile Glu Gly Ser Glu Val Ser Arg Val
            260                 265                 270

Thr Leu His Asn Glu Ser Tyr Ile Glu Glu Leu Asp Ile Arg Ile Gly
        275                 280                 285

Asp Trp Val Leu Val His Lys Ala Gly Gly Val Ile Pro Glu Val Leu
    290                 295                 300

Arg Val Leu Lys Glu Arg Arg Thr Gly Ala Glu Arg Pro Ile Val Trp
305                 310                 315                 320

Pro Glu Asn Cys Pro Glu Cys Gly His His Leu Val Lys Glu Gly Lys
                325                 330                 335

Val His Arg Cys Pro Asn Pro Leu Cys Pro Ala Lys Arg Phe Glu Ala
            340                 345                 350

Ile Arg His Tyr Ala Ser Arg Lys Ala Met Asp Ile Gln Gly Leu Gly
        355                 360                 365

Glu Lys Leu Ile Glu Lys Leu Leu Glu Asn Gly Leu Val Lys Asp Val
    370                 375                 380
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Ala Asp Leu Tyr Arg Leu Arg Lys Glu Asp Leu Val Gly Leu Glu Arg
385                 390                 395                 400

Met Gly Glu Lys Ser Ala Glu Asn Leu Leu Arg Gln Ile Glu Glu Ser
                405                 410                 415

Lys His Arg Gly Leu Glu Arg Leu Leu Tyr Ala Leu Gly Leu Pro Gly
            420                 425                 430

Val Gly Glu Val Leu Ala Arg Asn Leu Ala Ala Arg Phe Gly Thr Met
        435                 440                 445

Asp Arg Leu Leu Glu Ala Thr Leu Glu Glu Leu Leu Glu Val Glu Glu
    450                 455                 460

Val Gly Glu Leu Thr Ala Arg Gly Ile Trp Glu Thr Leu Gln Asp Pro
465                 470                 475                 480
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Ala.

Please replace the paragraph beginning at page 10, line 25, with the following paragraph:

The thermostable ligase of the present invention is also characterized by having an arginine adjacent to the active site lysine (i.e. K) in the KXDG (SEQ. ID. No. 24) motif (where X is any amino acid).

Please replace the paragraph beginning at page 26, line 3, with the following paragraph:

The oligonucleotide perfect match substrate was formed by annealing two short oligonucleotides (33-mer for LP3'C (SEQ. ID. No. 11) and 30-mer for Com3F (SEQ. ID. No. 12)) with a 59-mer complementary oligonucleotide (Glg). Oligonucleotides LP3'C and Glg (SEQ. ID. No. 14) were in 1.5-fold excess so that the all the 3' Fam labeled Com3F represented nicked substrates (see Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996), which is hereby incorporated by reference). The T/G mismatch substrate was formed by annealing LP3'T (SEQ. ID. No. 13), which introduced a single base-pair mismatch at the 3'-end of the nick junction, along with Com 3'F to the complementary strand (Glg). The nicked DNA duplex substrates were formed by denaturing DNA probes at 94°C for 2 min followed by re-annealing at 65°C for 2 min in ligation buffer. The sequences of the oligonucleotides were listed below (p represents 5' phosphate group):

(SEQ. ID. No. 12) pAGTTGTCATAGTTTGATCCTCTAGTCTGGG-FAM-3' Com3
LP3'T (SEQ. ID. No. 13) 5'- CCCTGTTCCAGCGTCTGCGGTGTTGCGTT
LP3'C (SEQ. ID. No. 11) 5'-AAAACCCTGTTCCAGCGTCTGCGGTGTTGCGTC
Glg (SEQ. ID. No. 14) 3'-GGGACAAGGTCGCAGACGCCACAACGCAGTCAACAGTATCAAACTAGGAGATCAGACCC-5'

Please replace the paragraph beginning at page 27, line 15, with the following paragraph:

Amino acid sequence alignment of five Gram negative bacterial NAD^+-dependent DNA ligases indicates that *Tth* ligase is 93% identical to *Thermus scotoductus* ligase, 49% to *Rhodothermus marinus* ligase, 48% to *E. coli* ligase, and 38% to *Zymomonas mobilis* based on sequence data retrieved from GeneBank. Degenerate primers corresponding to highly conserved regions of these ligases were used to amplify fragments of ligase genes from seven *Thermus* strains which represent a worldwide collection: *Thermus flavus* from Japan (SEQ. ID. No. 16), *Thermus aquaticus* YT-1 (SEQ. ID. No. 15) and *Thermus sp.* AK16D from Yellowstone National Park in the United States (SEQ. ID. No. 22), *Thermus filiformis* Tok4A2 (SEQ. ID. No. 17) and *Thermus filiformis* Tok6A1 (SEQ. ID. No. 18) from New Zealand, *Thermus sp.* SM32 (SEQ. ID. No. 19) from Azores, and *Thermus sp.* Vil3 (SEQ. ID. No. 20) from Portugal. The sequences of amplified ligase fragments ranging from 1.4 to 1.6 kb were determined by directly sequencing the PCR products using an ABI 373 automated sequencer. *Thermus* ligases, in general, were highly conserved during evolution as demonstrated by 85%-98% sequence identity. In contrast, the amino acid sequences of the restriction endonuclease *Taq*I and its isoschizomers from the identical strains show only 50-70% aa identities (Cao, et al., Gene, 197:205-214 (1997), which is hereby incorporated by reference). *Thermus* ligases in general show 30-40% sequence identities as compared with DNA ligases from other bacteria. The sequence divergence is slightly higher among the different geographic groups than within the same group, which may reflect random drift or adaptation to their respective local environments (Figure 1). *Thermus flavus*, *Thermus filiformis* Tok4A2, *Thermus filiformis* Tok6A1, *Thermus sp.* SM32, *Thermus sp.* Vil3, *Thermus aquaticus* YT-1, and *Thermus sp.* AK16D (SEQ. ID. No. ~~14~~ 1) ligases shared 98.2%, 89.9%, 89.5%, 89.8%, 88.3%, 88.2%, 88.1% with *Thermus thermophilus* HB8 DNA ligase, respectively. The adenylation site of the enzymes (^{118}KXDG (SEQ. ID. No. 24) where X is in general a hydrophobic residue), as identified by site-directed mutagenesis of *Tth* DNA ligase, is completely identical among all *Thermus* ligases, furthermore, the flanking sequences of the adenylation motif are also identical except *Tsp.* AK16D in which the aa residues ^{117}H before the ^{118}K is substituted by an ~~^{118}R~~ ^{117}R (Figure 1B). In non-*Thermus*

NAD^+-dependent ligases discovered to date, the corresponding position is either a Pro or a Leu. The two isolates from Japan can be distinguished from the other *Thermus* strains by a 3-aa-insertion at position 234.

Please replace the paragraph beginning at page 29, line 24, with the following paragraph:

Divalent metal ion is indispensable for each of the three steps in a ligation reaction: (i) adenylation of a lysine residue in the adenylation motif KXDG (SEQ. ID. No. 24); (ii) transfer of the adenylate to the 5' phosphate to form a DNA-adenylate intermediate; and (iii) formation of the phosphodiester bond with the release of adenosine monophosphate (AMP). In general, Mg^{2+} is the preferred metal ion for both ATP-dependent and NAD^+-dependent ligases. Mg^{2+} was substituted with alkaline earth metal ion Ca^{2+} and commonly studied period 4 transition metal ions. *Tth* and *Tsp.* AK16D ligases could use Mn^{2+} as an alternative metal cofactor to support ligation activity (Figure 4). Both enzymes were less active with Ca^{2+}, while Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} failed to support ligation. In comparison, ATP-dependent ligase from Hin (i.e. *Haemophilus influenzae*) uses only Mg^{2+} and Mn^{2+} as the metal cofactor for nick closure but not Ca^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} (Cheng, et al., Nucleic Acids Res, 25(7):1369-1374 (1997), which is hereby incorporated by reference); ATP-dependent ligase from *Chlorella* virus PBCV-1 can use Mg^{2+}, Mn^{2+}, and Co^{2+} but not Ca^{2+}, Cu^{2+}, and Zn^{2+} (Ho, et al., J Virol, 71(3):1931-1937 (1997), which is hereby incorporated by reference). Using Ca^{2+} as the metal cofactor, *Thermus* enzymes were able to convert most of the substrate into the DNA-adenylate intermediate. However, the rates of nick closure were reduced which led to the accumulation of the DNA-adenylate intermediate (Figure 4B). A small amount of the intermediate was observed with Ni^{2+}; however, ligation product was not observed at the current detection level, suggesting that Ni^{2+} could not support the nick closure step (Figure 4B). To further compare the relative activity of the two *Thermus* ligases with Mg^{2+} and Mn^{2+}, the generation of ligation product was first monitored over a 20-min time period. As shown in Figure 5, the *Thermus* enzymes were consistently more active with Mg^{2+} than with Mn^{2+}. Second, ligation activity up to 40 mM Mg^{2+} or Mn^{2+} concentrations (Figure 6) was assayed. Both of the enzymes responded sensitively to the change of the metal ion concentration in the reaction mixture. At high M^{2+} concentrations, the high ionic strength may inhibit the enzyme activity, consistent with KCl dependence profile (Figure 4).

Similar to the time-course results, the *Thermus* enzymes were more active with Mg^{2+} than with Mn^{2+} (Figure 6). The discrepancy on the relative activity of *Thermus* ligases between this study and an earlier report may be due to use here of cloned enzymes while the earlier work used purified native enzyme (Takahashi, et al., J Biol Chem, 259(16):10041-10047 (1984), which is hereby incorporated by reference).

Please replace the paragraph beginning at page 35, line 24, with the following paragraph:

Studies on *Tth* DNA ligase has deepened understanding of thermostable ligases and has reaffirmed the common theme of ligation – adenylation of ligase at the KXDG (SEQ. ID. No. 24) motif (Luo, et al., Nucleic Acids Res, 24(15):3079-3085 (1996), which is hereby incorporated by reference). This study reveals that *Thermus* ligases may differ from each other as to substrate specificity despite their highly identical primary protein sequences. A highly homologous structure can be anticipated from various *Thermus* ligases, but subtle local environments may dictate the probability of accepting a particular mismatch as the substrate. The fidelity of the *Thermus* ligases may be determined by multiple domains, multiple motifs and/or multiple sequence elements. In comparison of *Tth* and *Tsp.* AK16D ligases, one can find that although K294R (in an identical local environment, see Figure 1B) enhances the fidelity of *Tth* ligase (Luo, et al., Nucleic Acids Res, 24(15):3071-3078 (1996), which is hereby incorporated by reference), *Tsp.* AK16D ligase with a K in this position can still demonstrate superior mismatch discrimination. Additional sequence elements remain to be uncovered. The R substitution at the adjacent position to the KXDG (SEQ. ID. No. 24) motif may have an effect on the *Tsp.* AK16D ligase's specificity, because studies on *Chlorella* ligase has emphasized the importance of occupying AMP binding pocket for nick recognition (Sriskanda, et al., Nucleic Acids Res, 26(2):525-531 (1998)). The accumulation of DNA-adenylate intermediate with some divalent metal ions by *Tsp.* AK16D ligase asserts that the nick closure step of a ligation reaction can be sensitive to the selection of metal ions, gapped substrates and mismatch substrates. More structural and functional studies on *Tsp.* AK16D ligase could reveal how this enzyme achieves high fidelity with different substrates and different metal ions.